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Submitting Organization
W.R. GRACE & COMPANY

Contractor
LITTON BIONETICS INC

Document Title
MUTAGENICITY EVALUATION OF L-2001 (FINAL REPORT) WITH COVER LETTER DATED 011691

Chemical Category
1,6-DIISOCYANATOHEXANE (822-06-0)

CONTAINS NO CBI

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January 16, 1991

Environmental Protection Agency
Document Processing Center (TS-790)
Room L-100
Office of Toxic Substances
401 "M" Street S.W.
Washington, D.C. 20460

Attn: Health and Safety Reporting Rule (Notification/Reporting)

Please find attached 8(d) health and safety reports for mixtures processed containing toluene diisocyanate (CAS #26471-62-5), 4,4-Diphenylmethane diisocyanate (CAS #101-68-8) and 1,6-Diisocyanatohexane (CAS #822-06-0). Grace is submitting these reports for late filing since their submittal may have been subject to the isocyanates 10-year call-in of June 1, 1987.

We have reason to believe that some of these reports may have previously been submitted to EPA as attachments to PMN submissions. However, Grace is filing them as a precautionary measure to insure EPA's receipt.

These reports are being submitted for:

W. R. Grace & Co.-Conn.
Washington Research Center
7379 Route 32
Columbia, MD 21044

Sincerely,

J. W. Raksis

A:\JR91-013/lw

Attachments - 20

86910000638

Toluene Diisocyanate
26471-62-5
1,6-Diisocyanatohexane
822-06-0

MUTAGENICITY EVALUATION

OF

L-2001

FINAL REPORT

SUBMITTED TO

W.R. GRACE AND COMPANY
7379 ROUTE 32
COLUMBIA, MARYLAND 21044

SUBMITTED BY

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 20338

DECEMBER, 1977

REVISED OCTOBER 1978

Litton BIONETICS

TABLE OF CONTENTS

SPONSOR: W.R. Grace and Company

MATERIAL: L-2001

SUBJECT: FINAL REPORT MUTAGENICITY PLATE ASSAY

1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

2. MATERIALS

A. Test Compound

1. Date Received: October 10, 1977

2. Description: Amber liquid

B. Indicator Microorganisms

Salmonella typhimurium, strains: TA-1535 TA-98
TA-1537 TA-100
TA-1538

Saccharomyces cerevisiae, strain: D4

C. Activation System (Ames et al., Mutation Research 31:347, 1975)

1. Reaction Mixture

Component	Final Concentration/ml
TPN	4 µmoles
Glucose-6-phosphate	5 µmoles
Sodium phosphate (dibasic)	100 µmoles
$MgCl_2$	8 µmoles
KCl	33 µmoles
Homogenate fraction equivalent to 25 mg of wet tissue	0.1-0.15 ml 9,000 x g supernatant of rat liver

2. S-9 Homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.

2. MATERIALS (Continued)

D. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

ASSAY	CHEMICAL[a]	SOLVENT	PROBABLE MUTAGENIC SPECIFICITY
Nonactivation	Methylnitrosoguanidine (MNNG)	Water or Saline	BPS[b]
	2-Nitrofluorene (NF)	Dimethylsulfoxide[c]	FS[b]
	Quinacrine mustard (QM)	Water or saline	FS[b]
Activation	2-Anthramine (ANTH)	Dimethylsulfoxide[c]	BPS[b]
	2-Acetylaminofluorene (AAF)	Dimethylsulfoxide[c]	FS[b]
	8-Aminoquinoline (AMQ)	Dimethylsulfoxide[c]	FS[b]

[a] Concentrations given in Results Section

[b] BPS = Base-pair substitution

FS = Frameshift

[c] Previously shown to be nonmutagenic

E. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed and its concentration are recorded in the Results Section.

3. EXPERIMENTAL DESIGN

A. Plate Test (Overlay Method*)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, at least four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests, a minimum of four different concentrations of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the 9,000 x g liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

*Certain classes of chemicals known to be mutagens and carcinogens do not produce detectable responses using the standard Ames overlay method. Some dialkyl nitrosamines and certain substituted hydrazines are mutagenic in suspension assays, but not in the plate assay. Chemicals of these classes should be screened in a suspension assay.

CLIENT: W.R. Grace

Test started - 10/31/77

CHEMICAL NAME: L-2001

	CONCENTRATION µl/plate	INDICATOR ORGANISMS TA-1535	TA-1537	TA-1538	TA-98	TA-100	D4*
-S9	SOLVENT CONTROL	22	10	16	38	180	369
	POSITIVE CONTROL**	952	716	1413	1154	1344	1214
	0.001000 µl	25	9	15	25	149	247
	0.010000 µl	33	12	7	27	177	242
	0.100000 µl	32	8	7	35	241	322
	1.000000 µl	24	0	7	16	271	0
	5.000000 µl	16	0	18	17	173	0
+S9	SOLVENT CONTROL	19	19	19	36	200	487
	POSITIVE CONTROL***	189	238	579	900	1248	531
	0.001000 µl	16	12	10	27	241	352
	0.010000 µl	24	16	12	28	249	412
	0.100000 µl	17	15	12	27	238	369
	1.000000 µl	15	5	13	27	267	439
	5.000000 µl	16	4	13	24	282	431

*Try + convertants per plate

**TA-1535 EMS 10 µl/plate
TA-1537 QX 10 µg/plate
TA-1538 NF 10 µg/plate
TA-98 NF 10 µg/plate
TA-100 EMS 10 µl/plate
D4 EMS 10 µl/plate
Solvent DMSO 50 µl/plate

***TA-1535 ANTH 2.5 µg/plate
TA-1537 ANTH 2.5 µg/plate
TA-1538 ANTH 2.5 µg/plate
TA-98 ANTH 2.5 µg/plate
TA-100 ANTH 2.5 µg/plate
D4 ANTH 2.5 µg/plate
Solvent DMSO 50 µl/plate

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5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of *in vitro* microbial assays employing *Salmonella* and *Saccharomyces* indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

A. Toxicity Test Results

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically-induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 0.001 µl to 5 µl per plate. The compound was toxic to the strain TA-1537 at 1 and 5 µl per plate.

B. Nonactivation Test Results

The results of the tests conducted on the compound in the absence of a metabolic system were all negative.

C. Activation Test Results

The results of the tests conducted on the compound in the presence of the rat liver activation system were all negative.

D. Conclusions

The test compound, L-2001, did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered as not mutagenic under these test conditions.

Submitted by:

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6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and the cells are incubated in the overlay for 2 to 3 days, and a few cell divisions occur during the incubation period, the test is semi-quantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.

- The combined incubation of the compound and the cells in the overlay permits constant exposure of the indicator cells for 2 to 3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs several doses ranging over two or three log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced, and the compound will not appear to be mutagenic.

6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a

6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

D. Evaluation Criteria for Ames Assay

3. Pattern

given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

E. Relationship Between Mutagenicity and Carcinogenicity

It must be emphasized that the Ames Salmonella/microsome test is not a definitive test for chemical carcinogens. It is recognized, however, that correlative and functional relationships have been demonstrated between these two end points. The results of comparative tests on 300 chemicals by McCann et al. (Proc. Nat. Acad. Sci. USA, 72:5135-5139, 1975) show an extremely good correlation between results of microbial mutagenesis tests and in vivo rodent carcinogenesis assays.

All evaluation and interpretation of the data presented in this report are based only on the demonstration of or lack of mutagenic activity.

STANDARD OPERATING PROCEDURES

To ensure an accurate and reliable mutagenicity testing program, LBI instituted the following procedures:

- The test compound was registered in a bound log book recording the date of receipt, complete client identification, physical description and LBI code number.

- Complete records of weights and dilutions associated with the testing of the submitted material were entered into a bound notebook.

- Raw data information was recorded on special printed forms that were dated and initialed by the individual performing the data collection at the time the observations were made. These forms were filed as permanent records.

- All animal tissue S-9 preparations used in the activation tests were taken from dated and pretested frozen lots identified by a unique number. The S-9 preparations were monitored for uniformity and the information recorded.

CERTIFICATE OF AUTHENTICITY

THIS IS TO CERTIFY that the microimages appearing on this microfiche are accurate and complete reproductions of the records of U.S. Environmental Protection Agency documents as delivered in the regular course of business for microfilming.

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